Fibrinogen and Factor XIII Polymorphisms
Contribution to Cardiovascular Disease

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Objectives

- Polymorphisms shape the pattern of variability in the human genome
- Association of polymorphisms in the beta fibrinogen gene with the risk of arterial thrombosis
- Association of Factor XIII A-subunit gene (FXIIIval34Leu) accelerates thrombin activation of factor XIII
The Human Genome

- 3 billion chemical bases or letters strung in a sequence over 23 pairs of chromosomes
Polymorphisms

- Our individual genome are largely identical, but there are 10 million points in the sequence where our individual codes can vary
- These discrepancies are known as polymorphisms
Figure 1: SNP
A single-base difference found between chromosomes in the DNA sequences, i.e., genetic information, is referred to as a SNP (single nucleotide polymorphism).
Structure of a Gene

Gene (DNA) → Transcription → Primary transcript (RNA) → Splicing → Mature transcript (mRNA) → Protein synthesis → Protein
Genomic Polymorphisms: Stable Heritable Changes in Drug Sensitivity

- Can affect transcription of gene and mRNA stability (gene expression)
- Can affect translation and protein activity
- About 1.4 million polymorphisms (mostly SNPs) have been identified
- Are determinants of drug disposition, tumor response, and drug side effects

Blood groups: ABO genes code for

- a glycosyltransferase which adds N-acetylglactosamine to H-antigen on A allele red blood cell
- B allele adds N-acetylglactosamine with 2 a.a differences that alter specificity
- O allele has frameshift mutation
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Fibrinogen is a rod shaped beta-globulin glycoprotein produced predominantly in hepatocytes.
There is a HMW and a LMW type of fibrinogen in plasma: HMW is most abundant (70%) and has high affinity calcium binding sites. LMW (30%) resulting from loss of C-terminal of one A-alpha chain, is slower to polymerize.
Schematic Model of Native Fibrinogen

Fibrinogen is a symmetric dimeric structure consisting of three pairs of non-identical polypeptide chains $\alpha$, $\beta$ and $\gamma$. 
The amino acid terminal regions of all three chains form the central E-domain. The central E-domain is 15% of the mass of native fibrinogen.
The carboxyl terminal regions of all three chains form the two D-domains. The two D-domains account for 50% of the mass of native fibrinogen.
Fibrinogen mediates platelet aggregation
1. Fibrinogen blood concentration maintains blood fluidity

Fibrinogen concentration (normal range 2-4 grams/Liter) is critical in maintaining blood fluid because elevated fibrinogen levels increase blood viscosity, an identified risk factor for thrombosis.
Ancrod is derived from the venom of a snake.

Malaysian Pit Viper
Calloselasma rhodostoma
Blood viscosity following initiation of ancrod therapy.
Fibrinogen Gene Region
Interactions with nuclear proteins and interleukin 6 govern fibrinogen acute phase response and plasma levels.
<table>
<thead>
<tr>
<th>POSITIVE ACUTE PHASE REACTANTS</th>
<th>NEGATIVE ACUTE PHASE REACTANTS</th>
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</thead>
<tbody>
<tr>
<td>Fibrinogen</td>
<td>Albumin</td>
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<tr>
<td>Apha-1 antitrypsin</td>
<td>Transferrin</td>
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<tr>
<td>Haptoglobin</td>
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<tr>
<td>Ceruloplasmin</td>
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<tr>
<td>C reactive protein(CRP)</td>
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<td>C3 portion of complement</td>
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<td>Alpha-1 acid glycoprotein</td>
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<td>(orosomucoid)</td>
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<td>Serum amyloid A (SAA)</td>
<td></td>
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<td>Ferritin</td>
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</tbody>
</table>
Changes in fibrinogen quantity and quality

- Fibrinogen is an acute phase reactant and its production is increased by glucocorticoids and cytokine IL-6 and interferon gamma INF-
Factors that influence fibrinogen quality and quantity

- Beta gene polymorphisms provoke lower threshold acute phase reactions
- Acute phase reaction increases degree of phosphorylation from 24% to 60% in 24 hours and also elevates fibrinogen concentration
- Acute phase fibrinogen is cleaved at a faster rate by thrombin (old fibrinogen is less phosphorylated)
B-beta gene polymorphisms interactions with nuclear proteins and interleukin 6 provoke inappropriate fibrinogen acute phase response and elevated fibrinogen levels.
Polymorphisms in B-beta gene promoter region

- Cause chronically elevated fibrinogen levels that are an exaggerated response to IL-6 and nuclear proteins.
- Indirectly increase blood viscosity
- Increased blood viscosity contributes to intra-vascular clotting by altering blood rheology.
Altered fibrinogen molecules stick together (polymerize) and occlude blood vessels slowing or preventing the flow of blood. This is not thrombosis. It is similar to vascular occlusion by sickle cells. Thrombosis invariably follows untreated vascular occlusion.
Calcium contributes to the integrity of fibrinogen and has a protective action against plasminogen degradation. Fibrinogen variants (single or 2 a.a polymorphism) lacking in calcium binding sites have altered fibrin polymerization and manifest in excessive bleeding phenotype.
Fibrinolysis Mechanisms

Plasminogen, t-PA, and α-2-antiplasmin attach to fibrin by molecular interaction at lysine residues in the fibrinolysis proteins. Activation of t-PA by fibrin occurs in the α-chain. Activated t-PA cleaves plasminogen to plasmin on fibrin surfaces.
Fibrin I: non-covalent interaction between E- and D-domains
This type of fibrin is extremely susceptible to plasmin degradation.

Fibrin

D-domain  E-domain  D-domain
Fibrin Assembly: Influence of Sialic Acid

The amount of sialic acid influences the rate of fibrin polymerization; an increase in acidic charge delays polymerization.
Non-inherited regional fibrinogen variants that delay the rate of fibrin polymerization

- **Phosphorylation:** In the fetus and during acute phase reactions, 70% of the human $\alpha$-chain is phosphorylated at 2 serine residues.

- **Glycosylation:** In liver disease, an increase in sialic acid increases the acidic charge of fibrin and delays the rate of fibrin polymerization.
Fibrin Assembly: Inherited Genetically Abnormal Variants

• Several abnormal fibrinogen variants that cannot bind t-PA are characterized by persistence of venous or arterial occlusion by thrombi.

• Several abnormal fibrinogen variants produce fibrin fibers that are thinner and highly branched and more compact than normal fibrin fibers. The compactness with reduced permeability of the fibrin clot makes it resistant to plasmin degradation.
Altered clot structure in the healthy relatives of patients with premature coronary artery disease


One hundred male first degree relatives aged 65 years or less and free of personal history of CAD were enrolled in the study. Fibrin clots composed of dense fiber networks are found in young CAD patients and are confirmed to occur in relatives of such individuals.
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Factor XIII

- Factor XIII is a blood coagulation protein distributed both extracellularly (in plasma) and intracellularly (in megakaryocytes, platelets and placenta).
- It is a heterotetramer consisting of 2 identical proenzyme subunits ($A_2$) and 2 identical carrier protein subunits ($B_2$).
Factor XIII

- Thrombin releases the fibrin cross-linking activity by cleavage of a peptide bond in the presence of fibrinogen,
  - Fibrinogen lowers the concentration of thrombin required for cleavage of Factor XIII in vitro.
- Much of FXIII circulates in blood in association with fibrinogen. Concentration in plasma is 70nM. It has a half-life of 9-14 days.
Factor XIII, the precursor of a transglutaminase enzyme is activated by thrombin
ACTIVATION OF FACTOR pXIII

**pXIII**
A<sub>2</sub>B<sub>2</sub> tetramer
(320 kDa)

**XIIla**
A'<sub>2</sub>B<sub>2</sub> tetramer
(310 kDa)

**XIIla**
A*<sub>2</sub> dimer
(150 kDa)

- A + activation peptide
  (4 kDa each)

- thrombin
- fibrin
- Ca<sup>2+</sup>
- fibrin
- active site

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Fibrin I: non-covalent interaction between E- and D-domains
This type of fibrin is extremely susceptible to plasmin degradation.
Fibrinolysis Mechanisms
When crosslinked fibrin undergoes fibrinolysis, the peptides joining D- and E- domains are cleaved, leading to fibrin split products.
Val34Leu polymorphism of factor XIII

- Intracellular stability and plasma concentration of different factor XIII Val34Leu genotypes are identical.
- The release of the activation peptide (only 3 amino acids away from the thrombin cleavage site) proceeds significantly faster than its wild type val34 counterpart.
Factor XIII and thrombosis

- Newly recognized factor XIII polymorphisms very close to the thrombin cleavage site on the A-subunit enhance the rate of factor XIII activation by thrombin, resulting in the rapid cross-linking of a fibrin that is highly resistant to plasmin.
Factor XIII activity levels, specific activity within the normal population.

A. plasma levels

B. Factor XIII activity

C. Factor XIII activity per unit FXIII level (specific activity)

R Anwar, L Gallivan, et al

Genotype/phenotype correlations for coagulation factor XIII: specific normal polymorphisms are associated with high or low factor XIII specific activity. Blood 1999;93:897-905
A common coding polymorphism in the factor XII A-subunit gene (FXIIIVAL34LEU) affects cross-linking activity.
Lipoprotein(a) Inhibits Proteolytic Degradation by Plasmin

• Lipoprotein(a) is an LDL-like lipoprotein that contains an additional protein apo(a). The apo(a) gene is highly homologous to that of plasminogen.

• Apoprotein(a) functions as an inhibitor of plasminogen activation, preventing plasmin from occurring. Linus Pauling postulates that by preventing plasmin from occurring, apoprotein(a) is an important determinant for the evolutionary advantage of lipoprotein(a).
Exogenous Inhibitors of Plasmin-Induced Proteolysis are Synthetic Lysine Analogs that Bind Plasminogen Kringle 4.

- Essential amino acid L-lysine
- Tranexamic acid and ε-aminocaproic acid
FACTOR XIII

![Diagram of Factor XIII protein structure with labeled domains: Beta sandwich, Core domain, Barrel 1, Barrel 2.](image)